

## PROTEIN BINDING OF METABOLITES FOR SOME ENDOCRINE DISRUPTING CHEMICALS

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**Summary:** Endocrine disruption refers to the ability of chemicals to interfere with hormonal systems, and has raised considerable concern in recent years. Endocrine disruptive chemicals (EDCs) pose a documented risk to wildlife and have the potential to negatively influence human health. Many modern synthetic compounds are readily metabolized to more polar forms that often contain hydroxy groups. This presence of polar groups and aromatic moieties in the parent compound or metabolite can play an important role in the mechanism of endocrine disruption. In addition, phase II metabolism (e.g., glucuronidation) can also lead to deactivation of the endocrine properties. The aim of this work was to predict protein binding of metabolites for some endocrine disrupting chemicals.

**Key words:** endocrine disrupting chemicals, metabolites, protein binding

## ПРОТЕИННО СВЪРЗВАНЕ НА МЕТАБОЛИТИТЕ ПРИ НЯКОИ СЪЕДИНЕНИЯ С ЕСТРОГЕННА АКТИВНОСТ

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**Резюме:** През последните години, стана ясно, че подтискането на функциите на ендокринната система се дължи на способността на някои съединения да оказват влияние върху хормоналната обмяна. Натрупан е голям брой данни за вредния ефект на съединенията с естрогенна активност върху дивата природа и отрицателното влияние върху човешкото здраве. Много синтетични съединения метаболизират до по-полярни форми, които често съдържат хидроксилна група. Това присъствие на полярни групи и ароматна част на изходното съединение или метаболитите му може да изиграе важна роля в механизма на ендокринното действие. Освен това, фаза II на метаболизма (напр. глюкуронирането) може да доведе до деактивация на функциите на ендокринната система. Целта на настоящата работа е да се предскаже протеиновото свързване на метаболитите при някои съединения с естрогенна активност.

**Ключови думи:** съединения с естрогенна активност, метаболити, протеиново свързване

### Introduction

Since the middle of the last century, many industrial chemicals and pesticides have been introduced into the environment. Many of these compounds are chlorinated aromatic structures, which are not easily metabolized, resulting in bioaccumulation or biomagnification. This bioaccumulation process has caused unwanted side effects in nontarget species, particularly those at higher trophic levels in the food chain. With growing awareness of these problems, various

persistent halogenated compounds have gradually been banned from use. Since then, environmental levels in biota have decreased slowly during the last decades. As a replacement, new products have been developed that are less hydrophobic than organochlorines, and consequently do not accumulate in humans and wildlife. The capacity of an organism to metabolize a compound to more polar products is often considered to be a detoxification mechanism. Furthermore, the presence of polar hydroxy groups in parent compounds often prevents bioaccumulation. In addi-

tion, introduction of even more polar groups such as glucuronides or sulfates further increases the ability of an organism to eliminate the compound [11, 18].

A disadvantage of this approach is that the presence of hydroxy groups and complicated structures with aromatic moieties may bear resemblance to steroid structures. As a result, less bioaccumulative compounds such as alkylphenols, phthalate esters, and methoxychlor have shown biological activities similar to estrogens or androgens. These compounds can act as either (partial) agonists or antagonists for steroid receptors such as the estrogen receptor. Such interactions may have consequences for (sexual) development, reproduction, and the formation of hormone-dependent tumors. Thus, subtle differences among molecules, such as the presence or absence of an OH group, can lead to significant changes in their ability to bind to steroid receptors or inhibit steroidogenic enzymes. The presence of an OH and/or aromatic group plays a significant role in both steroid metabolism and receptor binding [19, 20]. Thus, biotransformation plays a significant part in the endocrine-disrupting properties of a compound. On the one hand, the introduction of an OH group may bioactivate the parent compound by forming a metabolite that can interact with a steroid receptor or steroidogenic enzyme. On the other hand, a rapid phase II metabolism producing glucuronides or sulfates helps the organism to eliminate the parent compound from the body, reducing the opportunity for adverse effects. The aim of this work was to predict protein binding of metabolites for some endocrine disrupting chemicals.

## Material and methods

### 1. OECD (Q)SAR Toolbox.

The OECD (Q)SAR Application Toolbox is software tool used in regulatory toxicology to fill gaps in (eco)toxicity data. They include different SAR and QSAR models for estimating (eco)toxicological endpoints. (Quantitative) Structure-Activity Relationships [(Q)SARs] are methods for estimating properties of a chemical from its molecular structure and have the potential to provide information on hazards of chemicals, while reducing time, monetary cost and animal testing currently needed [10].

### 2. Observed microbial metabolism.

Degradation pathways used by microorganism to obtain carbon and energy from 200 chemicals are stored in a special file format that allows easy computer access to catabolic information. The collection includes the catabolism of C1-compounds, aliphatic hydrocarbons, alicyclic rings, furans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, sulfonates, nitrates, nitro-derivatives, nitriles, and compounds containing more than one functional group. Most of pathways are related to aerobic conditions. Different sources including monographs, scientific articles and public web sites such as the UM-BBD [4] were used to compile the database.

### 3. Microbial metabolism simulator.

The original CATABOL simulator of microbial metabolism is implemented in the system [2, 3, 6]. Single pathway catabolism is simulated using the abiotic and enzyme-mediated reactions via the hierarchically ordered principal molecular transformations extracted from documented metabolic pathway database. The hierarchy of the transformations is used to control the propagation of the catabolic maps of the chemicals. The simulation starts with the search for match between the parent molecule and the source fragment associated with the transformation having the highest hierarchy. If the match is not found search is performed with the next transformation, etc. When the match is identified, the transformation products are generated. The procedure is repeated for the newly-formed products. Predictability (probability that the metabolite is observed, given that the metabolite is predicted) evaluated on the bases of documented catabolism for 200 chemicals stored in the database of "Observed microbial catabolism" is 83%.

**4. Ligand-binding pocket and interaction mechanisms.** The region of the receptor-binding site not occupied by the receptor and thus available to the ligands and their analogues is called Receptor-excluded volume (RExV) [16]. An attempt to determine the volume of this map was done by superimposing molecular skeletons of known-high affinity ligands [9]. Based on the models for the ligand-binding pocket one could anticipate that the binding affinity is conditioned by electronic and hydrophobic interactions. Besides the traditional two sites interacting with the

nucleophiles on A and D rings a third receptor site situated at one of the binding pockets (11 $\beta$ ) could interact with the ligand. These three sites are denoted as A, B and C and as anticipated overall  $A > B \approx C$ , i.e. the A site provides the strongest interaction energy, whereas B and C sites have approximately same strength [7]. Four electronic interaction mechanisms could be anticipated based on these three sites: A–B, A–C, A–B–C (AD) and A only [13].

## Results and Discussion

In conjunction with the nervous and immune systems, the endocrine system forms the main regulatory mechanism that controls different pivotal functions in the human or animal body. The messengers of the endocrine system are hormones that are synthesized and excreted at very low quantities from specialized glands and transported to the target organ(s) via the bloodstream. Hormones are transported in the blood in the free state or attached to carrier proteins and bind at the target organs to specialized hormone receptors on the cell surface or within the cell (nuclear receptors). This hormone-receptor complex then activates different cell or organ functions.

Estrogen receptor (ER) binding is a molecular initiating event much like protein binding [15] that may lead to a series of adverse outcomes, which are typically linked to reproductive and development hazards. It is an endpoint where several comprehensive databases exist, which has led to the development of several approaches for using (Q)SARs to predict ER-binding and possible subsequent endocrine disruption [1]. Popular among these are the “four phase” assessment that includes Comparative Molecular Field

Analysis (CoMFA) [17] and the Common Reactivity Pattern Approach (COREPA) [14]. The present work showed that the QSAR approach in the OECD (Q) SAR Toolbox has been used for identifying interaction mechanisms for some endocrine disruptors in Table 1.

Biotransformations are chemical reactions that are catalyzed by microorganisms in terms of growing or resting cells or that are catalyzed by isolated enzymes.

Biotransformation involves two stages: phase I, mainly involving enzymatic activity from the cytochrome P450 (CYP) family; and phase II, catalyzed by conjugation enzymes like glutathione S-transferase (GST), UDP-glucuronosyltransferase, and N-acetyltransferase (NAT). Phase I enzymes promote the activation of drugs and pro-carcinogens for the genotoxic electrophilic intermediaries. Meanwhile, phase II enzymes generally act as inactivating enzymes, that is, they catalyze the binding of intermediary metabolites to cofactors, transforming them into more hydrophilic products, thus facilitating their elimination [12]. Therefore, the coordinated expression and regulation of xenobiotic metabolizing enzymes (XMEs) in both phase I and phase II and their metabolic equilibrium in the cells of target organs can be important factors in determining susceptibility to cancer as related to exposure to carcinogens [8].

In our previous experimental work, we measured the percentage removal of substrate and reaction rate of endocrine disruptors through oxidation by immobilized on a polypropylene membrane enzyme lacase from *Trametes versicolor* under standard conditions [5].

**Table 1.** Observed values, predicted ER and protein binding of selected endocrine disruptors



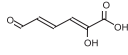
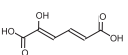
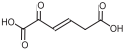
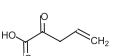
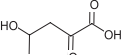




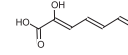
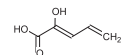
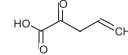
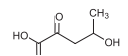
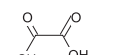



№	CAS number	Name of compounds	Observed values RBA, %	Predicted ER binding	Protein binding
1	108-95-2	Phenol	0	Weak binder, OH	No binding
2	80-05-7	Bisphenol A	0.008	Very strong binder, OH	No binding
3	136-83-4	2-Nonylphenol	-	Strong binder, OH	No binding
4	88-04-0	3,5-dimethyl-4-chlorophenol	-	Weak binder, OH	No binding
5	120-32-1	2-Benzyl-4-chlorophenol	-	Strong binder, OH	No binding
6	103-90-2	Paracetamol	-	Weak binder, OH	No binding

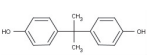
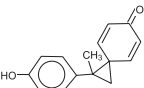
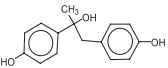
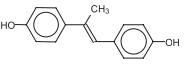
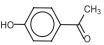
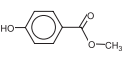
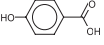
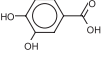

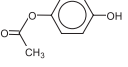

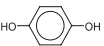

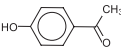
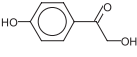
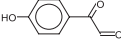
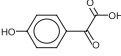



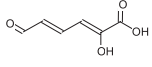
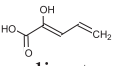
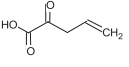
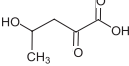


The listed values show that our immobilized laccase is able to oxidize a wide range of phenolic substrates. Taking as reference the phenol removal, i.e. the removal percentage of 40% after 30 min of enzyme treatment under standard conditions, it is possible to conclude that some endocrine disruptors (nonylphenol and chlorophene) are oxidized by laccase

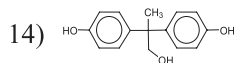
at a similar rate, other derivatives (paracetamol and chloroxyphenol) at a smaller rate, and others (bisphenol A) at higher rate.

Observed and predicted microbial metabolism, estrogenic activity and protein binding of some selected estrogenic compounds was predicted on the OECD (Q)SAR Toolbox (Table 2).

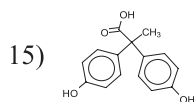
**Table 2.** Observed and predicted microbial metabolism of some selected endocrine disrupting chemicals, their estrogenic activity and protein binding.

№	CAS number	Name of compounds	Observed microbial metabolism/ Estrogenic activity; Protein binding	Predicted microbial metabolism/ Estrogenic activity; Protein binding
1	108-95-2	Phenol 	9 metabolites: 1)  weak binder, OH; No binding 2)  non-cyclic structure; Michael-type nucleophilic addition, Schiff base formation 3)  non-cyclic structure; No binding 4)  non-cyclic structure; Michael-type nucleophilic addition 5)  non-cyclic structure; No binding 6)  non-cyclic structure; No binding 7)  non-cyclic structure; Schiff base formation 8)  non-cyclic structure; No binding 9)  non-cyclic structure; No binding	10 metabolites: 1)  weak binder, OH; No binding 2)  non-cyclic structure; Michael-type nucleophilic addition, Schiff base formation 3)  non-cyclic structure; No binding 4)  non-cyclic structure; No binding 5)  non-cyclic structure; No binding 6)  non-cyclic structure; No binding 7)  non-cyclic structure; Schiff base formation 8)  non-cyclic structure; No binding 9) $\text{H}_2\text{C}=\text{O}$ non-cyclic structure; Schiff base formation 10)  non-cyclic structure; No binding

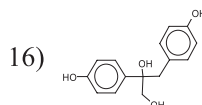
2	80-05-7	<p>Bisphenol A</p> 	<p>17 metabolites:</p> <p>1)  strong binder, OH; Michael-type nucleophilic addition</p> <p>2)  very strong binder, OH; No binding</p> <p>3)  very strong binder, OH; No binding</p> <p>4)  weak binder, OH; Nucleophilic addition to ketones</p> <p>5)  weak binder, OH; Arylcarboxylate aminolysis</p> <p>6)  weak binder, OH; No binding</p> <p>7)  weak binder, OH; No binding</p> <p>8)  non-cyclic structure; No binding</p> <p>9) <math>\text{H}_2\text{C}=\text{O}</math> non-cyclic structure; Schiff base formation</p> <p>10)  weak binder, OH; Arylcarboxylate aminolysis</p> <p>11)  non-cyclic structure; No binding</p> <p>12)  weak binder, OH; No binding</p> <p>13)  weak binder, OH; No binding</p>	<p>26 metabolites:</p> <p>1)  weak binder, OH; Nucleophilic addition to ketones</p> <p>2)  weak binder, OH; No binding</p> <p>3)  weak binder, OH; Schiff base formation</p> <p>4)  weak binder, OH; No binding</p> <p>5)  non-cyclic structure; No binding</p> <p>6)  weak binder, OH; No binding</p> <p>7)  weak binder, OH; No binding</p> <p>8)  non-cyclic structure; Michael-type nucleophilic addition, Schiff base formation</p> <p>9)  non-cyclic structure; No binding</p> <p>10)  non-cyclic structure; No binding</p> <p>11)  non-cyclic structure; No binding</p> <p>12)  non-cyclic structure; No binding</p> <p>13)  non-cyclic structure; Schiff base formation</p>
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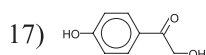
very strong binder, OH; No binding



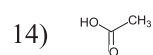
very strong binder, OH; No binding



very strong binder, OH; No binding



weak binder, OH; No binding



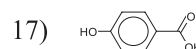
non-cyclic structure; No binding



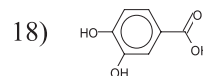
non-cyclic structure;  
Schiff base formation



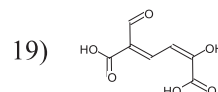
non-cyclic structure; No binding



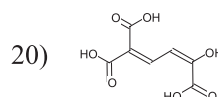
weak binder, OH; No binding



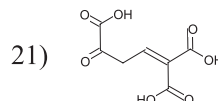
weak binder, OH; No binding



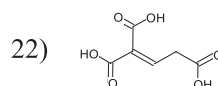
non-cyclic structure;  
Michael-type nucleophilic addition,  
Schiff base formation



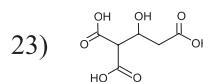
non-cyclic structure; No binding



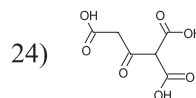
non-cyclic structure; No binding



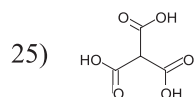
non-cyclic structure; No binding



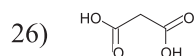
non-cyclic structure; No binding



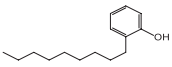
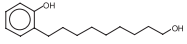
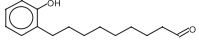
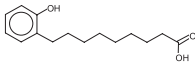
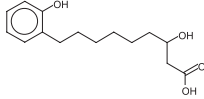
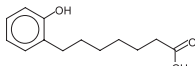
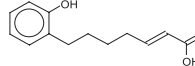
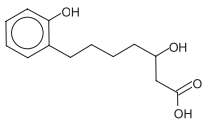
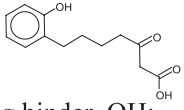
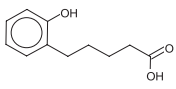
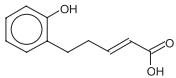
non-cyclic structure;  
Nucleophilic addition to ketones

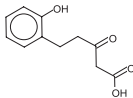
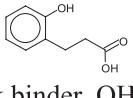
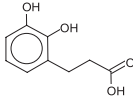
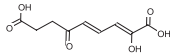
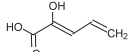
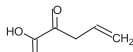
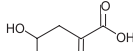
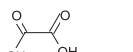
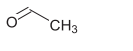
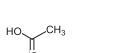
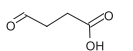
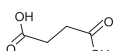
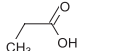


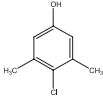

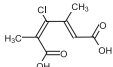
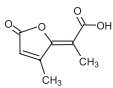

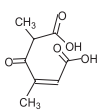


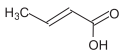
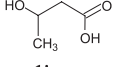


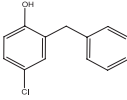
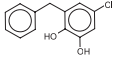
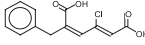
non-cyclic structure; No binding

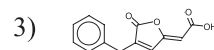


non-cyclic structure; No binding

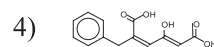
3	136-83-4	2-Nonylphenol 	0	<p>26 metabolites:</p> <p>1)  strong binder, OH; No binding</p> <p>2)  strong binder, OH; Schiff base formation</p> <p>3)  strong binder, OH; No binding</p> <p>4)  strong binder, OH; No binding</p> <p>5)  strong binder, OH; No binding</p> <p>6)  strong binder, OH; Nucleophilic addition to ketones</p> <p>7)  strong binder, OH; No binding</p> <p>8)  strong binder, OH; No binding</p> <p>9)  strong binder, OH; No binding</p> <p>10)  strong binder, OH; Nucleophilic addition to ketones</p> <p>11)  Moderate binder, OH; No binding</p> <p>12)  Moderate binder, OH; No binding</p> <p>13)  strong binder, OH; No binding</p>
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- 14)   
strong binder, OH;  
Nucleophilic addition to ketones
- 15)   
weak binder, OH; No binding
- 16)   
Moderate binder, OH; No binding
- 17)   
non-cyclic structure;  
Michael-type nucleophilic addition
- 18)   
non-cyclic structure; No binding
- 19)   
non-cyclic structure; No binding
- 20)   
non-cyclic structure; No binding
- 21)   
non-cyclic structure; No binding
- 22)   
non-cyclic structure;  
Schiff base formation
- 23)   
non-cyclic structure; No binding
- 24)   
non-cyclic structure;  
Schiff base formation
- 25)   
non-cyclic structure; No binding
- 26)   
non-cyclic structure; No binding

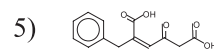
4	88-04-0	<p>3,5-dimethyl-4-chlorophenol</p> 	0	<p>11 metabolites:</p> <p>1)  Moderate binder, OH; No binding</p> <p>2)  non-cyclic structure; No binding</p> <p>3)  Without OH or NH<sub>2</sub> group; No binding</p> <p>4)  non-cyclic structure; No binding</p> <p>5)  non-cyclic structure; Michael-type nucleophilic addition</p> <p>6)  non-cyclic structure; No binding</p> <p>7)  non-cyclic structure; No binding</p> <p>8)  non-cyclic structure; No binding</p> <p>9)  non-cyclic structure; No binding</p> <p>10)  non-cyclic structure; Nucleophilic addition to ketones</p> <p>11)  non-cyclic structure; No binding</p>
5	120-32-1	<p>2-Benzyl-4-chlorophenol</p> 	0	<p>22 metabolites:</p> <p>1)  strong binder, OH; No binding</p> <p>2)  Without OH or NH<sub>2</sub> group; No binding</p>



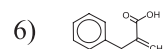
Without OH or NH<sub>2</sub> group;  
No binding



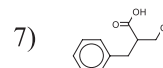
Without OH or NH<sub>2</sub> group;  
No binding



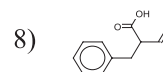
Without OH or NH<sub>2</sub> group;  
Michael-type nucleophilic addition



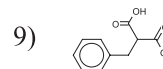
Without OH or NH<sub>2</sub> group;  
No binding



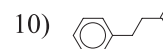
Without OH or NH<sub>2</sub> group;  
No binding



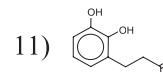
Without OH or NH<sub>2</sub> group;  
Schiff base formation



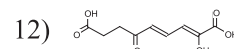
Without OH or NH<sub>2</sub> group;  
No binding



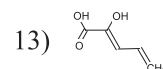
Without OH or NH<sub>2</sub> group;  
No binding



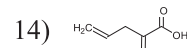
Moderate binder, OH; No binding



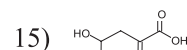
non-cyclic structure;  
Michael-type nucleophilic addition






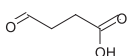
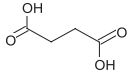


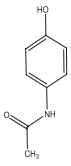
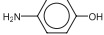

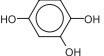
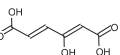
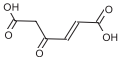



non-cyclic structure; No binding



non-cyclic structure; No binding



non-cyclic structure; No binding

				<p>16) </p> <p>non-cyclic structure; No binding</p> <p>17) </p> <p>non-cyclic structure; Schiff base formation</p> <p>18) </p> <p>non-cyclic structure; No binding</p> <p>19) </p> <p>non-cyclic structure; Schiff base formation</p> <p>20) </p> <p>non-cyclic structure; No binding</p> <p>21) </p> <p>non-cyclic structure; No binding</p> <p>22) </p> <p>non-cyclic structure; No binding</p>
6	103-90-2	<p>Paracetamol</p> 	0	<p>8 metabolites:</p> <p>1) </p> <p>weak binder, NH2; weak binder, OH; No binding</p> <p>2) </p> <p>weak binder, OH; No binding</p> <p>3) </p> <p>weak binder, OH; No binding</p> <p>4) </p> <p>non-cyclic structure; No binding</p> <p>5) </p> <p>non-cyclic structure; Michael-type nucleophilic addition</p> <p>6) </p> <p>non-cyclic structure; No binding</p> <p>7) </p> <p>non-cyclic structure; No binding</p> <p>8) </p> <p>non-cyclic structure; No binding</p>

Protein binding for all parent compounds is observed to be no binding but some of metabolites with estrogenic activity have been demonstrated protein binding (Table 2). For example, bisphenol A is not bound to protein but the first observed metabolite binds to protein by Michael-type nucleophilic addition.

## Conclusion

Metabolism plays a significant role in bioactivation and/or deactivation of endocrine active substances (EASs). The presence of hydroxy or keton groups and aromatic moieties play an important role in the mechanism of action of EASs. To prevent persistence and bioaccumulation/magnification, many modern synthetic compounds are readily metabolized to more polar forms often containing one or more hydroxyl groups. This enhanced ability to undergo biotransformation also has increased the potential for the formation of more EASs. The additional and consistent testing of metabolites for endocrine-disrupting properties should be encouraged in the future in order to establish a better risk assessment process for these types of compounds.

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